Proteasome inhibitors as new anticancer drugs

Julian Adams, PhD

The targeted degradation of key regulatory proteins is an essential element of cell cycle control. The proteasome plays a central role in the degradation of such proteins and has therefore become an important therapeutic target for diseases involving cell proliferation, notably cancer. This review summarizes numerous studies demonstrating that proteasome inhibition induces apoptosis and sensitizes cancer cells to traditional tumoricidal agents both *in vitro* and *in vivo*. The potent and selective proteasome inhibitor, PS-341, is particularly promising from a therapeutic perspective, and it is the only such inhibitor that has progressed to clinical trials. Preliminary data indicate that the drug is well tolerated by patients with cancer, and further trials are underway to assess the safety and efficacy of proteasome inhibition in hematologic and solid tumors, both as a monotherapy and in combination with other chemotherapeutics. Curr Opin Oncol 2002, 14:628–634

Millennium Pharmaceuticals, Cambridge, Massachusetts, USA.

Correspondence to Julian Adams, PhD, Millennium Pharmaceuticals, 35 Landsdowne Street, Cambridge, MA 02139, USA; e-mail: jadams@mpi.com

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Abbreviations

MTD	maximum tolerated dose
NFκB	nuclear factor κB

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The 26S proteasome, a multicatalytic enzyme expressed in the nucleus and cytoplasm of all eukaryotic cells, has traditionally been viewed as a recycler of damaged or misfolded proteins, but it has become increasingly apparent over the last decade that the proteasome plays a vital role in degrading numerous proteins [1] that regulate the cell cycle and perhaps apoptosis. Proteasome substrates include transcription factors, signaling molecules, tumor suppressors, cell cycle regulators, and inhibitory molecules such as IκB and securin, among others [2]. Targeted protein degradation might also be required not only for the normal progression of the cell cycle but also for the accelerated and uncontrolled mitosis characteristic of cancer development and spread [3]. Inhibition of the proteasome may therefore arrest or retard cancer progression by interfering with the ordered degradation of cell cycle proteins [4••]. Indeed, the inhibition of proteasomal function results in programmed cell death (apoptosis) [5].

This review summarizes recent studies that have examined the use of proteasome inhibitors for the treatment of cancer.

Ubiquitin–proteasome pathway

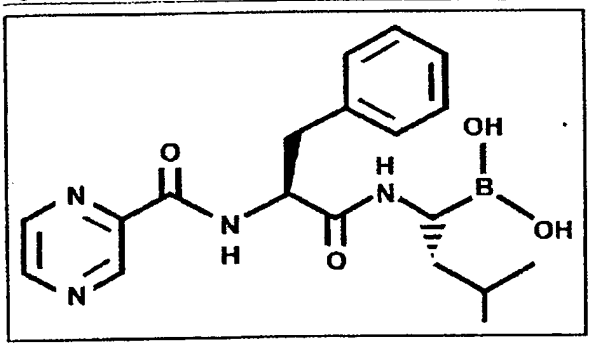
The targeting of proteins for proteasomal degradation is a highly regulated process. The primary mechanism by which proteins are directed to the proteasome involves the attachment of a polyubiquitin chain [6]. The first step of the ubiquitin–proteasome pathway is the covalent attachment of a single ubiquitin molecule to a free amino group on the target protein. The selectivity of protein targeting is largely conferred by the enzymes that perform this initial reaction [7•]. In subsequent reactions, further ubiquitin molecules are added to form the polyubiquitin chain. It is this chain that is recognized by the proteasome.

Structure of the proteasome

The 26S proteasome is a multiprotein complex consisting of a 20S core particle associated with one or two 19S regulatory particles (Fig. 1). The 19S subunit binds the polyubiquitin chain and cleaves it from the protein substrate. The substrate is then denatured, allowing access to the proteolytic core [2].

The 20S core particle is a barrel-shaped structure composed of four stacked rings in an α-β-β-α conformation. The outer α rings complex with the 19S regulatory particles, forming a narrow channel through which only de-

Figure 1. Schematic of 26S proteasome



The 26S proteasome is a 2000-kD multiprotein complex composed of a proteolytically active 20S core particle that is capped by one or two 19S regulatory particles. These regulatory particles recognize ubiquitinated proteins and control access to the proteolytic core. Published with permission from Millennium Pharmaceuticals, Cambridge, MA.

natured proteins may pass. The catalytic chamber is formed by the two inner β rings, each of which contains three active sites. These active sites account for the three major proteolytic activities of the proteasome: chymotrypsin-like, trypsin-like, and postglutamyl peptide hydrolase-like [8]. Proteins are degraded by the core particle in a progressive manner, generating peptides three to 25 residues in length [9].

Proteasome inhibitors

Numerous proteasome inhibitors have been described, many of which interfere directly with the proteolytic activity of the 20S core particle. These inhibitors bind either reversibly or irreversibly to active sites in the core particle, and they display varying levels of specificity for the 26S proteasome.

The synthetic peptide aldehydes, including Z-Leu-Leu-Leu-H (MG-132) and Z-Ile-Glu(OBut)Ala-Leu-H (PSI), were among the first proteasome inhibitors to be described [10]. These compounds reversibly inhibit the chymotrypsin-like activity of the proteasome such that protein degradation is restored upon their removal [4••]. However, they are also potent inhibitors of thiol proteases such as cathepsin B and the calpains, and they may display poor metabolic stability and bioavailability [10,11].

Lactacystin is a natural compound that hydrolyzes in aqueous solution to form *clasto*-lactacystin β-lactone, an inhibitor of all three proteolytic activities of the proteasome [12]. This inhibition is irreversible for trypsin-like and chymotrypsin-like activities [13]. Although β-lactone is more selective for the proteasome than the aforementioned peptide aldehydes [14], it does exhibit some activity against cathepsin A [15]. Furthermore, the inhibitor is unstable because β-lactone is itself hydrolyzed to form an inactive metabolite [12].

Synthetic peptides possessing a carboxyl vinyl sulfone (VS) moiety are irreversible inhibitors of the proteasome. Peptides such as Z-Leu-Leu-Leu-VS (NLVS) are relatively easily synthesized and covalently block all three proteolytic activities of the proteasome [16]. However, vinyl sulfone-based inhibitors have limitations similar to those of the peptide aldehydes in that they bind and inhibit cathepsin S [16].

Natural epoxyketone-containing compounds such as epoxomicin and eponemycin are potent and irreversible proteasome inhibitors [17]. Epoxomicin primarily inhibits the chymotrypsin-like activity of the proteasome [17], whereas eponemycin and its synthetic analogue dihydroeponemycin inhibit chymotrypsin-like and postglutamyl peptide hydrolase-like activity at comparable rates [18]. These agents are extremely selective proteasome inhibitors, with no activity against any other protease [17].

The TMC-95s are a class of cyclic peptides that inhibit the proteasome in a reversible manner. These compounds form hydrogen bonds with the protein backbone of the proteasome, sterically hindering access to the active sites [8]. The structure of the 20S proteasome–TMC-95A complex has recently been elucidated, and TMC-95A is seen as a model for the development of synthetic inhibitors suitable for clinical use [8].

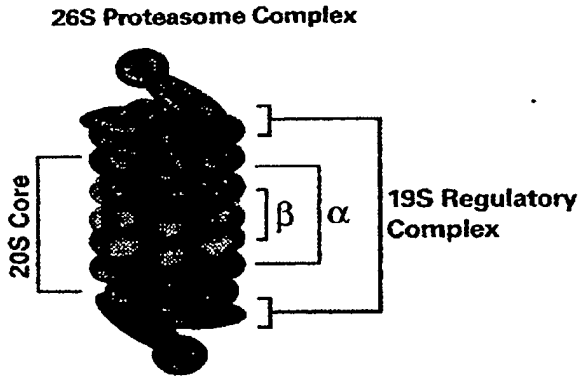
From a clinical perspective, perhaps the most promising proteasome inhibitors are the peptide boronic acids. These compounds are as much as 1000-fold more potent than their aldehyde analogues and are extremely selective for the proteasome over common serine proteases [10]. The peptide boronic acids block the chymotrypsin-like activity of the proteasome in a reversible manner and dissociate from the proteasome at a slower rate than the peptide aldehydes. Within this new class of peptides, the dipeptidyl boronic acids offer the additional advantages of relatively low molecular weight and simplicity of synthesis [10].

The compact, water-soluble dipeptidyl boronic acid PS-341 is of particular interest (Fig. 2). PS-341 is an extremely potent (K_i = 0.6 nM) and selective proteasome inhibitor. It binds to the proteasome with very high affinity and dissociates slowly, conferring stable but reversible proteasome inhibition [10]. These characteristics make PS-341 especially suitable for clinical use.

Activity of proteasome inhibitors *in vitro*

Proteolysis by the 26S proteasome is a fundamental metabolic process, and the complete blockade of proteasomal activity results in programmed cell death [5]. Importantly, however, antitumor activity can be achieved with less than maximal inhibition, and tumor cells seem to be more sensitive to proteasome inhibitors than are normal cells. For example, multiple myeloma cells [19]

Figure 2. Structure of dipeptidyl boronic acid proteasome inhibitor PS-341



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and leukemic cells [20] are significantly more sensitive to the proapoptotic effects of proteasome inhibition than are CD34(+) bone marrow progenitor cells or lymphocytes from healthy people.

Actively dividing cells are also more sensitive to proteasome inhibition than are quiescent or differentiated cells. Thus, human leukemia cells that have been induced to differentiate are significantly less sensitive to the apoptotic effects of PSI than their rapidly proliferating precursors [21]. Similarly, quiescent endothelial cells are less sensitive to proteasome inhibitor-induced apoptosis than are dividing cells [22]. However, it is unlikely that whether the hypersensitivity of cancer cells to proteasome inhibition can be explained solely by their accelerated rate of division.

Proteasome inhibition also increases the sensitivity of cancer cells to traditional anticancer agents such as gemcitabine [23], cisplatin [20,24,25], paclitaxel [24], irinotecan (CPT-11) [26•], and radiation [27,28]. Indeed, proteasome inhibition is sufficient to overcome the resistance of some cell types to conventional therapies [29,30]. For example, proteasome inhibition sensitizes murine lymphoma cells previously resistant to radiation-induced apoptosis, despite elevated expression of Bcl-2 [30]. Similarly, high doses of PS-341 induce apoptosis in MIA-PaCa-2 human pancreatic cancer cells, whereas low doses increase the cytotoxicity of gemcitabine [23].

The precise mechanisms by which proteasome inhibitors induce cell death are yet to be established. Although the stabilization of p21, p27, and p53 are common responses to proteasome inhibition [26,30,31•,32,33], the involvement of these regulatory proteins in apoptosis varies between cell types. Thus, proteasome inhibitor-induced apoptosis is dependent upon p53 expression in some cell lines [5,30] but not in others [19,26,29,32–34]. Current theories of apoptosis suggest that the initiation of programmed cell death is not mediated by any one protein, but rather by the ratio of antiapoptotic to proapoptotic proteins within a cell [35]. Proteasome inhibition may therefore induce apoptosis, or increase sensitivity to apoptosis, by disturbing this balance.

Effects on cell cycle-regulatory proteins and transcription factor nuclear factor κB

The effects of proteasome inhibition on the stability of various cell cycle regulatory proteins, including cyclins (*eg*, cyclin B1), cyclin-dependent kinase inhibitors (*eg*, p21 and p27) [31•], tumor suppressors (*eg*, p53), and the transcription factor nuclear factor κB (NFκB), are well established [36–38]. The last is of particular interest, because interference with the NFκB transcriptional pathway appears to influence tumor cell survival [39•].

Under normal circumstances, NFκB is sequestered in the cytoplasm and rendered inactive by the inhibitor protein IκB. In times of cell stress, however, IκB is degraded by the proteasome, and NFκB translocates to the nucleus. NFκB promotes cell survival by initiating the transcription of genes encoding stress-response enzymes, cell-adhesion molecules, proinflammatory cytokines, and antiapoptotic proteins such as Bcl-2, cIAP1, and cIAP2 [40–43]. NFκB is constitutively active in certain malignancies [44,45] and has been shown to promote tumor cell survival and reduce the effectiveness of anticancer therapy [37,46]. The constitutive activity of NFκB may also be correlated with *in vitro* drug resistance [47].

Proteasome inhibition has been shown to block the chemotherapy-induced activation of NFκB, resulting in enhanced chemosensitivity and increased apoptosis in cancer cells [26•,48]. Similarly, inhibition of NFκB activation increases radiation-induced apoptosis and enhances the radiosensitivity of colorectal cancer cells *in vitro* and *in vivo* [28].

Cytotoxic effects on tumor cell lines

Several proteasome inhibitors have demonstrated antitumor activity *in vitro*. For example, lactacystin and MG-132 potently induce apoptosis in B cells isolated from patients with B-cell chronic lymphocytic leukemia [49]. These agents also induce apoptosis in cultured p53-defective leukemic cells [34] and in several human malignant glioma lines [50]. Similarly, MG-132 has been shown to induce apoptosis in HD-My-Z Hodgkin cells and in p53-competent and p53-defective gastric cancer cells [32].

A standard National Cancer Institute screen of 60 cancer cell lines derived from multiple human tumors has shown PS-341 to have potent anticancer activity *in vitro* [33]. PS-341 was found to penetrate cancer cells and

inhibit the proteolysis of long-lived proteins, with 50% proteolytic inhibition observed at concentrations of approximately 0.1 μM. Further, PS-341 potently inhibited the growth of these cells, with an average dose of only 7 nM required to achieve 50% growth inhibition. Importantly, a comparison of the cytotoxic profile of PS-341 with the historical results of 60,000 other compounds found it to be unique, with little similarity to other "standard" or investigational agents [33]. Additional studies have subsequently confirmed the cytotoxicity of PS-341 in human MCF-7 breast carcinoma cells [51] and BxPC3 pancreatic cancer cells [31•].

There are important indicators that these *in vitro* results will be relevant to tumor growth *in vivo*. PS-341 is as effective against multicellular spheroid cultures as against monolayer cultures and thus may show promising activity against solid tumors with low growth fractions [52]. Furthermore, PS-341 induces apoptosis in proliferating endothelial (human umbilical vein endothelial) cells [53] and prevents vascularization in an embryonic chick chorioallantoic membrane system [22], suggesting that proteasome inhibition may limit angiogenesis *in vivo*.

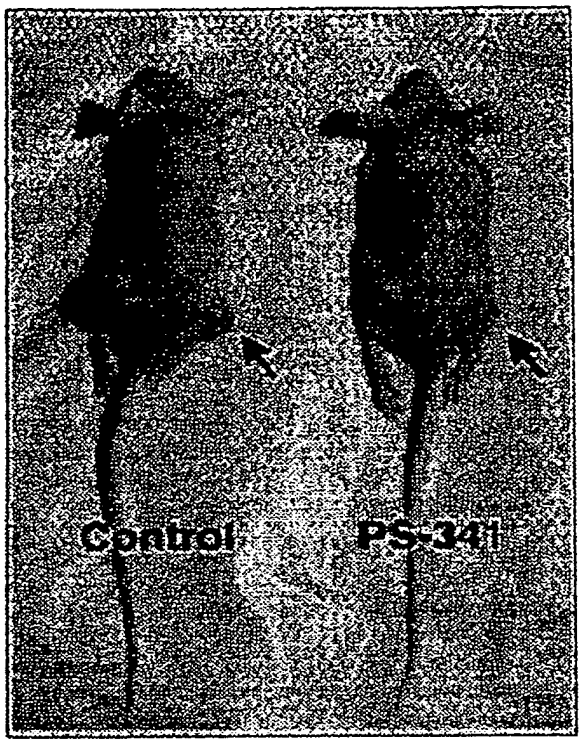
Activity of proteasome inhibitors in tumor-bearing animal models

PS-341 is the only proteasome inhibitor to have been extensively studied in xenograft models. Preliminary data indicate that PS-341 has significant activity against human xenografts of multiple myeloma [54], mantle cell lymphoma-xenografted severe combined immunodeficiency mice [55], pancreatic cancer [23,31•] colon cancer [26•], prostate cancer [33], and squamous cell carcinoma [39•], with some animals exhibiting complete tumor regression.

In concurrence with *in vitro* studies, proteasome inhibition appears to increase the potency of traditional tumoricidal agents in an additive or synergistic manner in xenograft models. PS-341 dramatically inhibits tumor growth in murine xenograft models of human pancreatic cancer when used in combination with gemcitabine [23] or CPT-11 [31•] and human colon cancer when used in combination with CPT-11 therapy [26•]. PS-341 also significantly inhibits tumor growth in colon cancer-xenografted mice receiving ionizing radiation (Fig. 3) [28].

Similarly, proteasome blockade inhibits the growth of grafted murine prostate tumors treated with radiation [27] and grafted murine mammary tumors treated with cisplatin, cyclophosphamide, or radiation [51]. Moreover, PS-341 alone and in combination with other tumoricidal agents is highly effective against lung metastatic disease in mice [51].

Figure 3. Proteasome inhibition increases potency of traditional tumoricidal agents



PS-341 treatment in combination with radiotherapy significantly reduces the growth of colorectal cancer xenografts in mice. The mouse on the left received a single injection of saline, whereas the mouse on the right received a single injection of PS-341 (1 mg/kg). Tumors on the right flank of both animals were then irradiated (6 Gy). The combination of PS-341 and radiation has a greater tumoricidal effect than either PS-341 or radiation alone. Published with permission [28].

Histologic analysis indicates that tumor growth inhibition in murine xenografts is accompanied by an increase in cancer cell apoptosis. Treatment with PS-341 alone is sufficient to induce apoptosis in human mantle cell lymphoma xenografts [55], whereas combined PS-341 and CPT-11 therapy yields highly apoptotic cells in mice bearing human colon [26•] or pancreatic tumors [31•]. Histologic analysis of blood vessel density also reveals that, in addition to its direct cytotoxic effects, PS-341 appears to have antiangiogenic activity in tumors [39•].

PS-341 acts in a dose-dependent manner in xenograft models and is well tolerated in doses as high as 0.5 or 1.0 mg/kg body weight [26,28,54]. Studies of drug distribution after intravenous injection indicate that, whereas most organs receive a similar amount of the drug, PS-341 is present at relatively low levels in the skin. Thus, subcutaneous tumors would have limited exposure to the drug, and the potency of PS-341 may be underestimated

in this model system [33]. Proteasome inhibition was not detected in the central nervous system, eyes, or testes, suggesting that PS-341 does not readily cross tight endothelial cell junctions [33].

Studies are underway to investigate the properties of xenografted tumors grown from PS-341-resistant multiple myeloma cells [56].

Proteasome inhibitors as new cancer drugs in clinical trials

PS-341 is the only proteasome inhibitor to have progressed to clinical trials. More than 30 clinical trials sponsored by Millennium Pharmaceuticals, the Cancer Therapy Evaluation Program of the National Cancer Institute, or independent investigators are evaluating the use of PS-341 in patients with hematologic malignancies or solid tumors. Approximately 200 patients have thus far received the drug in phase I studies, with an additional 256 in phase II myeloma trials and numerous others in the Cancer Therapy Evaluation Program.

Because PS-341 is cleared rapidly from the plasma compartment, traditional pharmacokinetic parameters have not proven useful for dose optimization in phase I trials. A novel bioassay has therefore been used as a guide to dose escalation in phase I studies. This assay rapidly and reliably measures residual proteasome activity in whole blood or white blood cells, and it may also be used in tissue biopsies to quantify the effect of the drug in solid tumors. Bioassay testing in volunteers indicated minimal intersubject and intrasubject variation in proteasome activity. In rats, bioassay testing and whole-body autoradiography after administration of (^{14}C)PS-341 indicate that most organs receive approximately the same amount of the drug [33].

The effects of PS-341 are clearly dose-dependent. Toxicology studies in rodents and primates show that the primary adverse events associated with PS-341 treatment are anorexia, vomiting, and diarrhea. These gastrointestinal adverse events are dose-related, with PS-341 generally well tolerated to as much as 80% proteasome blockade [33].

A single maximum tolerated dose (MTD) has not been established in clinical trials. Indeed, preliminary data indicate that MTD may vary by tumor type and treatment schedule. Phase I trials in solid tumors are assessing escalating doses of PS-341 in combination with common chemotherapeutic agents such as gemcitabine, irinotecan, and docetaxel, among others. Phase II trials in hematologic malignancies are currently exploring doses of 1.3 mg/m^2 twice weekly for 2 weeks, with 1 week of rest. Preliminary analysis of one of these trials [57] revealed that, of 78 patients analyzed, approximately 7% of patients responded (complete, partial, or minor), including 20% of patients with more than 90% reduction in paraprotein, and 30% had stable disease. A randomized multicenter international phase III trial comparing PS-341 with dexamethasone in patients with relapsed disease began in the first half of 2002.

Based on the results in xenograft experiments using combination therapy, PS-341 is being combined with numerous standard agents in dose-ranging phase I trials. To date, the trials indicate that the combination of PS-341 and gemcitabine or irinotecan has manageable toxicities [58,59]. Based on the results of the PS-341 and gemcitabine phase I trial in advanced solid tumors, the recommended regimen to be assessed in phase II trials of pretreated patients is 1.0 mg/m^2 PS-341 and 1000 mg/m^2 gemcitabine [58]. This trial continues to enroll chemotherapy-naïve patients to determine the MTD of this combination in patients who have not received previous chemotherapy. MTD in the PS-341 and irinotecan trial has not yet been reached [59]. Future phase II studies are planned.

Conclusions

During the last year, numerous preclinical studies have demonstrated that proteasome inhibition has a direct antitumor effect and sensitizes cancer cells to anticancer agents in current use. The mechanism by which proteasome inhibition induces apoptosis is under investigation, and these studies provide a rationale for further examination of proteasome inhibitors as potential therapeutics. Preliminary clinical trials in patients with cancer indicate that PS-341 is well tolerated at doses significantly inhibiting proteasome activity, and that further studies are justified.

In addition to its antitumor effects, proteasome inhibition may prove effective in the treatment of conditions involving inflammation [60], because many of the molecules that mediate inflammation are affected by proteasomal degradation. The proteasome is therefore an attractive target for drug development, and PS-341 is the first in a potentially promising therapeutic class.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • Of special interest
- •• Of outstanding interest

1 Rock KL, Gramm C, Rothstein L, et al.: Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. Cell 1994, 78:761–771.

2 Zwickl P, Voges D, Baumeister W: The proteasome: a macromolecular assembly designed for controlled proteolysis. Philos Trans R Soc Lond B Biol Sci 1999, 354:1501–1511.

3 Kumatori A, Tanaka K, Inamura N, et al.: Abnormally high expression of proteasomes in human leukemic cells. Proc Natl Acad Sci U S A 1990, 87:7071–7075.

4 •• Shah SA, Potter MW, Callery MP: Ubiquitin proteasome pathway: implications and advances in cancer therapy. Surg Oncol 2001, 10:43–52.

A concise review of the advances in proteasome inhibition to mid-2001. Includes details of specific proteasome targets and the role of the proteasome in apoptosis and chemoresistance.

5 MacLaren AP, Chapman RS, Wyllie AH, et al.: p53-dependent apoptosis induced by proteasome inhibition in mammary epithelial cells. Cell Death Differ 2001, 8:210–218.

6 Wilkinson KD: Ubiquitin-dependent signaling: the role of ubiquitination in the response of cells to their environment. J Nutr 1999, 129:1933–1936.

7 Pickart CM: Mechanisms underlying ubiquitination. Annu Rev Biochem 2001, 70:503–533.
A comprehensive review of ubiquitination, focusing on the enzymes involved in protein targeting.

8 Groll M, Koguchi Y, Huber R, et al.: Crystal structure of the 20 S proteasome:TMC-95A complex: a non-covalent proteasome inhibitor. J Mol Biol 2001, 311:543–548.

9 Nussbaum AK, Dick TP, Keilholz W, et al.: Cleavage motifs of the yeast 20S proteasome beta subunits deduced from digests of enolase 1. Proc Natl Acad Sci U S A 1998, 95:12504–12509.

10 Adams J, Behnke M, Chen S, et al.: Potent and selective inhibitors of the proteasome: dipeptidyl boronic acids. Bioorg Med Chem Lett 1998, 8:333–338.

11 Kisselev AF, Goldberg AL: Proteasome inhibitors: from research tools to drug candidates. Chem Biol 2001, 8:739–758.

12 Dick LR, Cruikshank AA, Grenier L, et al.: Mechanistic studies on the inactivation of the proteasome by lactacystin: a central role for clasto-lactacystin beta-lactone. J Biol Chem 1996, 271:7273–7276.

13 Fenteany G, Standaert RF, Lane WS, et al.: Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by lactacystin. Science 1995, 268:726–731.

14 Tomoda H, Omura S: Lactacystin, a proteasome inhibitor: discovery and its application in cell biology. Yakugaku Zasshi 2000, 120:935–949.

15 Kozlowski L, Stoklosa T, Omura S, et al.: Lactacystin inhibits cathepsin A activity in melanoma cell lines. Tumor Biol 2001, 22:211–215.

16 Bogyo M, McMaster JS, Gaczynska M, et al.: Covalent modification of the active site threonine of proteasomal beta subunits and the Escherichia coli homolog HslV by a new class of inhibitors. Proc Natl Acad Sci U S A 1997, 94:6629–6634.

17 Meng L, Mohan R, Kwok BH, et al.: Epoxomicin, a potent and selective proteasome inhibitor, exhibits in vivo antiinflammatory activity. Proc Natl Acad Sci U S A 1999, 96:10403–10408.

18 Meng L, Kwok BH, Sin N, et al.: Eponemycin exerts its antitumor effect through the inhibition of proteasome function. Cancer Res 1999, 59:2798–2801.

19 Ma MH, Parker KM, Manyak S, et al.: Proteasome inhibitor PS-341 markedly enhanced sensitivity of multiple myeloma cells to chemotherapeutic agents and overcomes chemoresistance through inhibition of the NF-κB pathway [abstract]. Blood 2001, 98:473a.

20 Soligo D, Servida F, Delia D, et al.: The apoptogenic response of human myeloid leukaemia cell lines and of normal and malignant haematopoietic progenitor cells to the proteasome inhibitor PSI. Br J Haematol 2001, 113:126–135.

21 Drexler HC: Activation of the cell death program by inhibition of proteasome function. Proc Natl Acad Sci U S A 1997, 94:855–860.

22 Drexler HC, Risau W, Konerding MA: Inhibition of proteasome function induces programmed cell death in proliferating endothelial cells. FASEB J 2000, 14:65–77.

23 Bold RJ, Virudachalam S, McConkey DJ: Chemosensitization of pancreatic cancer by inhibition of the 26S proteasome. J Surg Res 2001, 100:11–17.

24 Oyaizu H, Adachi Y, Okumura T, et al.: Proteasome inhibitor 1 enhances paclitaxel-induced apoptosis in human lung adenocarcinoma cell line. Oncol Rep 2001, 8:825–829.

25 Li QQ, Yunmbam MK, Zhong X, et al.: Lactacystin enhances cisplatin sensitivity in resistant human ovarian cancer cell lines via inhibition of DNA repair and ERCC-1 expression. Cell Mol Biol (Noisy-le-Grand) [serial online] 2001, 47:OL61–OL72.

26 Cusack JC Jr, Liu R, Houston M, et al.: Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: implications for systemic nuclear factor-kappaB inhibition. Cancer Res 2001, 61:3535–3540.
A good example of proteasome inhibition enhancing the chemosensitivity of cancer cells both *in vitro* and *in vivo*. Discusses the role of NFκB in chemoresistance.

27 Pervan M, Pajonk F, Sunday JR, et al.: Molecular pathways that modify tumor radiation response. Am J Clin Oncol 2001, 24:481–485.

28 Russo SM, Tepper JE, Baldwin AS Jr, et al.: Enhancement of radiosensitivity by proteasome inhibition: implications for a role of NF-kappaB. Int J Radiat Oncol Biol Phys 2001, 50:183–193.

29 Hideshima T, Richardson P, Chauhan D, et al.: The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Res 2001, 61:3071–3076.

30 Kurland JF, Meyn RE: Protease inhibitors restore radiation-induced apoptosis to Bcl-2- expressing lymphoma cells. Int J Cancer 2001, 96:327–333.

31 Shah SA, Potter MW, McDade TP, et al.: 26S proteasome inhibition induces apoptosis and limits growth of human pancreatic cancer. J Cell Biochem 2001, 82:110–122.
A detailed study demonstrating the direct apoptotic effect of PS-341 *in vitro* and the chemosensitizing effect of PS-341 *in vivo*. This work is of particular interest given the aggressive nature of pancreatic cancer and the lack of effective therapy to date.

32 Fan XM, Wong BC, Wang WP, et al.: Inhibition of proteasome function induced apoptosis in gastric cancer. Int J Cancer 2001, 93:481–488.

33 Adams J, Palombella VJ, Sausville EA, et al.: Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res 1999, 59:2615–2622.

34 Naujokat C, Sezer O, Zinke H, et al.: Proteasome inhibitors induced caspase-dependent apoptosis and accumulation of p21WAF1/Cip1 in human immature leukemic cells. Eur J Haematol 2000, 65:221–236.

35 Liotta LA, Liu ET: Essentials of molecular biology: basic principles. In Cancer: Principles and Practice of Oncology, 6th ed. Edited by DeVita VT, Hellman S, Rosenberg SA. Philadelphia: Lippincott Williams & Wilkins; 2001.

36 An WG, Hwang SG, Trepel JB, et al.: Protease inhibitor-induced apoptosis: accumulation of wt p53, p21WAF1/CIP1, and induction of apoptosis are independent markers of proteasome inhibition. Leukemia 2000, 14:1276–1283.

37 Jeremias I, Kupatt C, Baumann B, et al.: Inhibition of nuclear factor kappaB activation attenuates apoptosis resistance in lymphoid cells. Blood 1998, 91:4624–4631.

38 Wu Y, Luo H, Kanaan N, Wu J: The proteasome controls the expression of a proliferation-associated nuclear antigen Ki-67. J Cell Biochem 2000, 76:596–604.

39 Sunwoo JB, Chen Z, Dong G, et al.: Novel proteasome inhibitor PS-341 inhibits activation of nuclear factor-kappa B, cell survival, tumor growth, and angiogenesis in squamous cell carcinoma. Clin Cancer Res 2001, 7:1419–1428.
A study demonstrating that PS-341 as a single agent induces tumor regression and reduces blood vessel density *in vivo*.

40 Chen C, Edelstein LC, Gelinas C: The Rel/NF-kappaB family directly activates expression of the apoptosis inhibitor Bcl-x(L). Mol Cell Biol 2000, 20:2687–2695.

41 Wang CY, Mayo MW, Korneluk RG, et al.: NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 1998, 281:1680–1683.

42 Wang CY, Guttridge DC, Mayo MW, et al.: NF-kappaB induces expression of the Bcl-2 homologue A1/Bfl-1 to preferentially suppress chemotherapy-induced apoptosis. Mol Cell Biol 1999, 19:5923–5929.

43 Zong WX, Edelstein LC, Chen C, et al.: The prosurvival Bcl-2 homolog Bfl-1/A1 is a direct transcriptional target of NF-kappaB that blocks TNFalpha-induced apoptosis. Genes Dev 1999, 13:382–387.

44 Tricot G: New insights into role of microenvironment in multiple myeloma. Lancet 2000, 355:248–250.

45 Kordes U, Krappmann D, Heissmeyer V, et al.: Transcription factor NF-kappaB is constitutively activated in acute lymphoblastic leukemia cells. Leukemia 2000, 14:399–402.

46 Patel NM, Nozaki S, Shortle NH, et al.: Paclitaxel sensitivity of breast cancer cells with constitutively active NF-kappaB is enhanced by IkappaBalpha super-repressor and parthenolide. Oncogene 2000, 19:4159–4169.

47 Um JH, Kang CD, Lee BG, et al.: Increased and correlated nuclear factor-kappa B and Ku autoantigen activities are associated with development of multidrug resistance. Oncogene 2001, 20:6048–6056.

48 Cusack JC, Liu R, Baldwin AS: NF-kappa B and chemoresistance: potentiation of cancer drugs via inhibition of NF-kappa B. Drug Resist Updat 1999, 2:271–273.

49 Almond JB, Snowden RT, Hunter A, et al.: Proteasome inhibitor-induced apoptosis of B-chronic lymphocytic leukaemia cells involves cytochrome c release and caspase activation, accompanied by formation of an approximately 700 kDa Apaf-1 containing apoptosome complex. Leukemia 2001, 15: 1388–1397.

50 Wagenknecht B, Hermisson M, Groscurth P, et al.: Proteasome inhibitor-induced apoptosis of glioma cells involves the processing of multiple caspases and cytochrome c release. J Neurochem 2000, 75:2288–2297.

51 Teicher BA, Ara G, Herbst R, et al.: The proteasome inhibitor PS-341 in cancer therapy. Clin Cancer Res 1999, 5:2638–2645.

52 Frankel A, Man S, Elliott P, et al.: Lack of multicellular drug resistance observed in human ovarian and prostate carcinoma treated with the proteasome inhibitor PS-341. Clin Cancer Res 2000, 6:3719–3728.

53 Kumeda SI, Deguchi A, Toi M, et al.: Induction of G1 arrest and selective growth inhibition by lactacystin in human umbilical vein endothelial cells. Anticancer Res 1999, 19:3961–3968.

54 LeBlanc R, Catley L, Hideshima T, et al.: Proteasome inhibitor PS-341 inhibits human multiple myeloma cell growth in a murine model [abstract]. Blood 2001, 98:774a.

55 Pham L, Tamayo A, Lo P, et al.: Antitumor activity of the proteasome inhibitor PS-341 in mantle cell lymphoma B cells [abstract]. Blood 2001, 98:465a.

56 Steiner P, Neumeier H, Lightcap ES, et al.: Generation of PS-341-adapted human multiple myeloma cells as experimental tools for analysis of proteasome function in cancer [abstract]. Blood 2001, 98:310a.

57 Richardson PG, Berenson J, Irwin D, et al.: Phase II study of PS-341, a novel proteasome inhibitor, alone or in combination with dexamethasone, in patients with multiple myeloma who have relapsed following front-line therapy and are refractory to their most recent therapy [abstract]. Blood 2001, 98:774a.

58 Ryan DP, Eder JP, Winkelmann J, et al.: Pharmacokinetic and pharmacodynamic phase I study of PS-341 and gemcitabine in patients with advanced solid tumors. Proc Am Soc Clin Oncol 2002, 21:95a.

59 Clark JW, Ryan D, Dees C, et al.: Phase I dose-escalation study of the proteasome inhibitor, PS-341, plus irinotecan in patients with advanced solid tumors. Proc Am Soc Clin Oncol 2002, 21:93a.

60 Palombella VJ, Conner EM, Fuseler JW, et al.: Role of the proteasome and NF-kappaB in streptococcal cell wall-induced polyarthritis. Proc Natl Acad Sci U S A 1998, 95:15671–15676.